An injection moulded microfluidic chip for polymerase chain reaction (PCR) thermo-cycling and imaging of droplets to detect food-borne pathogens Campylobacter spp

Poulsen, Carl Esben; Bang, Dang Duong; Dufva, Martin; Wolff, Anders

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

DTU Nanotech
Department of Micro- and Nanotechnology

An injection moulded microfluidic chip for polymerase chain reaction (PCR) thermo-cycling and imaging of droplets to detect food-borne pathogens *Campylobacter* spp

C. E. Poulsen, D. D. Bang, M. Dufva & A. Wolff

Introduction

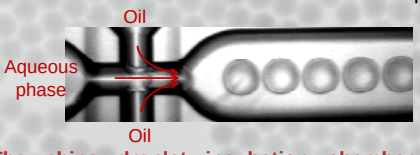
***Campylobacter* is a dangerous but common bacteria** which each year account for an increasing amount of cases of campylo-bacteriosis[1]. The low bacterial dose required to infect and its potentially fatal consequences make this organism a serious threat to the public health in both the developed and developing countries[2]. In poultry, the infection is often asymptomatic and chickens may hence carry the disease to the age of slaughtering, effectively contaminating the slaughter house[2].

To date, most droplet microfluidic chips are fabricated in poly(dimethylsiloxane) (PDMS) bonded to glass slides due to the ease of fabrication and surface modification[3]. This fabrication method is, however, not applicable to mass production and the potential for commercialisation is therefore reduced. **Alternatively, injection moulding** and hot embossing of thermoplastics are of interest.

Methods

Water-in-oil droplets are produced by a flow focusing chip. A PCR sample is injected into the aqueous line using a HPLC injection loop.

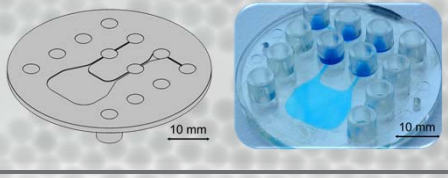
The droplets are collected in an injection moulded disposable all-polymer chip which has been bonded (sealed) using ultra sonic welding. This results in a turnaround time ~1 minute/chip.



The chips droplet incubation chamber is designed to exploit the positive buoyancy of the droplets to facilitate optimal droplet packing regardless of the droplet production rate and water-to-oil flow-rate ratio. The height of the chamber restricts the droplets to pack into a single layer. This monolayer enables fluorescence read out by fluorescence microscopy.

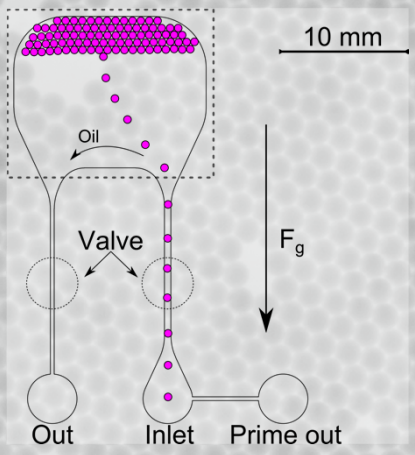
Droplet size and fluorescence intensity is extracted from the images using a custom MATLAB script which performs a circular hough transformation to identify droplets.

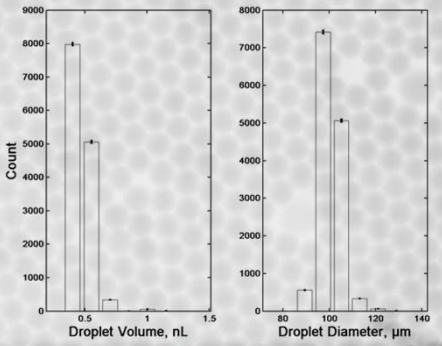
Thermocycling is conducted on a flat bed PCR machine controlled by a custom made LabVIEW script.



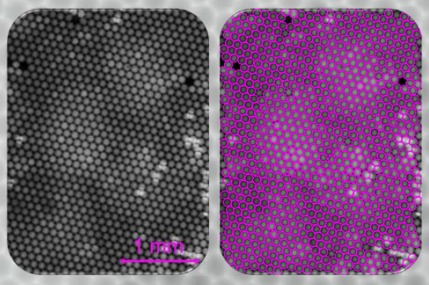
Results

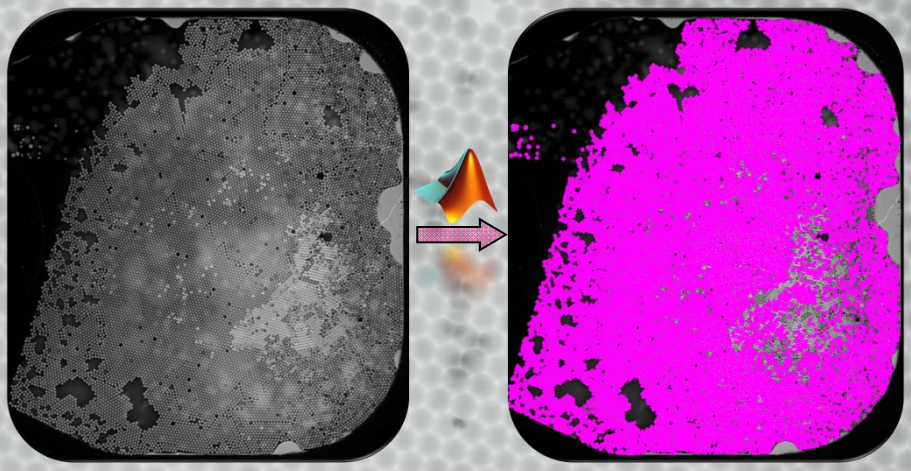
A single image contains as many as 20.000 droplets which may be analysed all at once. The droplets are highly uniform and forms almost exclusively a single densely packed monolayer.





Amplification by on-chip droplet PCR was confirmed by gel electrophoresis.





Conclusion

A disposable all-polymer multipurpose droplet interogation chip has been fabricated and demonstrated. Using a standard fluorescence microscope, thousands of droplets could be analysed using a single snap-shot. On-chip thermo-cycling of droplets for digital PCR was demonstrated and PCR product was detected.

1. D. D. Bang, E. M. Nielsen, F. Scheutz, K. Pedersen, K. Handberg, and M. Madsen, "PCR detection of seven virulence and toxin genes of Campylobacter jejuni and Campylobacter coli isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates.," Journal of applied microbiology, vol. 94, no. 6, pp. 1003–14, Jan. 2003.
2. J. E. Moore, D. Corcoran, J. S. G. Dooley, S. Fanning, B. Lucey, M. Matsuda, D. A. McDowell, F. Mégraud, B. C. Millar, R. O'Mahony, L. O'Riordan, M. O'Rourke, J. R. Rao, P. J. Rooney, A. Sails, and P. Whyte, "Campylobacter.," Veterinary research, vol. 36, no. 3, pp. 351–82, 2005.
3. S.-Y. Teh, R. Lin, L.-H. Hung, and A. P. Lee, "Droplet microfluidics.," Lab on a chip, vol. 8, no. 2, pp. 198–220, Feb. 2008.

DTU Nanotech
Department of Micro- and Nanotechnology